FABRICATION AND CHARACTERIZATION OF BACTERIA CELLULOSE, POLYVINYL ALCOHOL AND FRAGRANT OIL WITH ANTIMICROBIAL EFFECT FOR WOUND HEALING APPLICATION

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Thesis submitted in partial fulfillment of the requirements for the degree of

Bachelor of Chemical Engineering (Biotechnology)

February 2013

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ABSTRACT

The experiment is about the production of the wound healing product from the combination of three types of materials that is bacteria cellulose, polyvinyl alcohol (PVA) and also essential oil. The objective of the experiment is to fabricate the bacteria cellulose, polyvinyl alcohol (PVA) and essential oil with antibacterial properties for wound healing application. The method of the process can be divides into three that is preparation of bacterial cellulose, the fabrication of bacterial cellulose with PVA and essential oil and lastly characterization the product produced. In the fabrication process, the bacterial cellulose will be added with 1:4, 1:2, 3:4, and 1:1 ratios of PVA to BC. *Acetobactor Xylinum* is the bacteria that had been use to produce the bacteria cellulose. Among the method that will be used to characterize the product are Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), gas pycnometer, water absorption test and also antibacterial effect test. The entire tests are to make sure that the biocomposite film has the characteristics need as the wound healing material. The final product is in the form of film that is suitable to be used as the wound healing application. The result for the experiment is the higher ratios of PVA to BC content in the mixture of bacterial cellulose and essential oil will show the highest amount of the bond in the film. So, the present of the high amount of molecular bond inside the film will make it stronger. Then, for the SEM test, the higher ratios of PVA to BC content in the film will give the smooth morphology, homogenous and compact structure to the film. For moisture absorption test, the higher ratios of PVA to BC content will make the film can absorb more moisture compared to less amount of PVA content. For bacteria test, the higher ratios of PVA to BC content is more effective to kill more bacteria and lastly for the density test, the higher ratios of PVA to BC content in the film will give the higher density to the film. As the recommendation, use gram positive bacteria for the next study to see the effect of the biocomposite film with different ratio of PVA to BC content to the gram positive bacteria. Beside, try to produce the fragrant oil by yourself t to make sure the purity of the oil and also use

the combination of several fragrant oil to know the effectiveness of it antimicrobial effect.

FABRIKASI DAN PENCIRIAN SELULOSA BACTERIA, POLYVINYL ALCOHOL DAN MINYAK PATI DENGAN KESAN ANTIMIKROB UNTUK APLIKASI PENYEMBUHAN LUKA

ABSTRAK

Eksperimen ialah mengenai pengeluaran produk penyembuhan luka daripada gabungan tiga jenis bahan-bahan yang selulosa bakteria (SB), polyvinyl alkohol (PVA) dan juga minyak pati lavender. Objektif eksperimen adalah untuk menghasilkan selulosa bakteria, alkohol polyvinyl (PVA) dan minyak pati lavender dengan ciri-ciri antibakteria untuk penyembuhan luka. Kaedah pemprosesan terbahagi kepada tiga iaitu penyediaan selulosa bakteria, fabrikasi selulosa bakteria dengan PVA dan minyak pati dan diakhiri dengan pencirian produk yang dihasilkan. Dalam proses fabrikasi, selulosa bakteria akan ditambah dengan 1:4, 1:2, 3:04, dan 01:01 nisbah PVA terhadap SB. *Acetobactor xylinum* adalah bakteria yang telah gunakan untuk menghasilkan selulosa bakteria. Antara kaedah yang akan digunakan untuk mencirikan produk ialah Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy(SEM), piknometer gas, ujian penyerapan air dan juga ujian kesan antibakteria. Ujian keseluruhan adalah adalah untuk pastikan bahawa filem biokomposit mempunyai ciri-ciri yang diperlukan sebagai bahan penyembuhan luka. Produk akhir adalah dalam bentuk filem yang sesuai untuk digunakan sebagai bahan penyembuhan luka. Keputusan eksperimen menunjukkan nisbah kandungan PVA terhadap SB yang lebih tinggi dalam campuran selulosa bakteria dan minyak pati akan menunjukkan jumlah ikatan antara molecul yang tertinggi dalam filem. Jadi, kehadir jumlah yang ikatan molekul tinggi di dalam filem itu akan menjadikan ia lebih kuat. Kemudian, untuk ujian SEM, nisbah kandungan PVA terhadap SB yang lebih tinggi dalam filem akan memberi morfologi yang halus, struktur yang homogenus dan padat kepada filem. Untuk ujian penyerapan lembapan, nisbah kandungan PVA terhadap SB yang lebih tinggi akan membuat filem itu boleh menyerap lebih kelembapan berbanding dengan jumlah yang kurang kandungan nisbah PVA terhadap SB. Untuk ujian bakteria, nisbah kandungan PVA terhadap SB yang lebih tinggi adalah lebih berkesan untuk membunuh lebih banyak bakteria dan akhir sekali untuk ujian kepadatan, nisbah kandungan PVA terhadap SB yang lebih

tinggi dalam filem itu akan memberikan ketumpatan yang lebih tinggi kepada filem. Jesteru itu disyorkan penggunaan bakteria gram positif untuk kajian seterusnya bagi melihat kesannya terhadap filem biokomposit dengan nisbah kandungan PVA terhadap SB yang berbeza terhadap bakteria gram positif. Disamping itu, disyorkan juga untuk menghasilkan minyak pati secara sendiri bagi memastikan ketulenan minyak pati dan juga menggunakan gabungan minyak pati untuk mengetahui keberkesanan nya terhadap kesan antibajteria.

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LIST OF NOMENCLATURES

^oC	degree Celcius
BC	bacterial cellulose
CFU	colony forming units
EAA	Effectiveness antibacterial activity
FTIR	Fourier transform infrared spectroscopy
Mm	millimeter
µL	micrometer
PVA	polyvinyl alcohol
SEM	scanning electron microscopy

CHAPTER 1

INTRODUCTION

1.0 Background Of Study

Skin is the biggest organ of the human body. Its act as the barrier against the environment and also the first barrier of defend for human body from pathogen and infection. If the skin is damaged or destroyed, the moisture content and protein from the skin will be lost and will caused the infection to the damage area or wound to be increased. When the area of the wound increased, bacteria or pathogen can easily enter the human body that can caused internal infection. Healing of skin wound is complex process which requires the involvement of many different tissues, cell types and also matrix component (Martin *et al.*, 2007; Balasubramaniam *et al*., 2001)

Wound dressing is the method use by person as the application of the wound healing. It usually used in the first aid and nursing. Wound dressing is different from bandage because bandage only function to make sure dressing is at its place. Dressing was design to be directly having contact with wound. Historically, wound dressing was made by a piece of material such as cloth, cobwebs, dug, leaves and also honey. In the 1960s, that gauze and other absorbent materials (e.g. cotton) were passive products that “plugged and concealed” the wound but did little to encourage wound healing resulted in the creation of a minimal set of criteria for an ideal dressing (Turner 2001).Nowadays, modern wound dressings include gauze, film, foams, hydrogel, polysaccharide pastes, granules and beads. According to Turner (1979), the idea wound dressing need to have the ability to absorb exudates and toxic components from the wounds surface, maintain a high humidity at the wound dressing interface, allow gaseous exchange, provide thermal insulation, protect the wound from bacterial penetration, be nontoxic and also can be remove easily without trauma to the wound.

Bacterial cellulose or also known as biocellulose is the cellulose that produced by bacteria. It is usually been used traditionally in the food industries especially in producing *nata-de-coco*. This food product is native to Philippines and unconventional based on the fermentation of the coconut water (Jagannath *et al.*, 2008). The bacteria used in the production of the bacteria cellulose is the *Acetobacto xylinum* (Yamada *et al.*, 2000). This bacteria can extracellulary synthesized the cellulose into nano-sized fibril. The latest application of the bacteria cellulose used is as the material in the medical application. Due to their great characteristics such as good in mechanical properties, water sorption capacity, porosity, stability and also conformability, it’s have been reported used as an artificial skin for the person that had extensive wound that cause by burns, artificial blood vessel (Klemm *et al.*, 2005), tissue engineering of cartilage (Svensson *et al.*, 2005), wound dressing (Legeza *et al.*, 2004) and also wound healing (Czaja *et al.*, 2006)

The fragrant oil or also known as the essential oil, aromatic oil, ethereal oil and also steam volatile oil. Fragrant oil have been made from plant from the process of distillation and it is concentrated and easily to volatile. Before, the uses of the fragrant oil are only for the fragrant and also the flavor. But the uses of it had expended into pharmaceutical industries with the introduction of aromatherapy that well known that can give relaxation to the practitioner because of its ability to stimulate the sense. Beside it also have the ability to prevent infection that have been cause by microorganism such as bacteria and virus because it's naturally have an antibacterial properties (Aromaweb, 2012).

Polyvinyl alcohol (PVA) is representative of a water soluble synthetic polymer. The raw material used in the manufacture of polyvinyl alcohol is vinyl acetate monomer and it's involving the process of polymerization of vinyl acetate followed by partial hydrolysis. It is odorless, tasteless, translucent, and nontoxic. Polyvinyl alcohol is excellence in film forming, emulsifying and adhesive. Beside polyvinyl alcohol also resistances to oil, grease and solvents. However all their properties are depend on the humidity. The physical properties and specific functional uses of the polyvinyl alcohol are depending on the degree of polymerization and the degree of the hydrolysis. Low temperature crystalized PVA has light transparency and nutrition permeability (Kobayashi *et al*., 1992).

Biocomposite are formed from the natural fibers that usually from plants. Cellulose is one of the bicomposite that formed from plant and consist of biodegradable fibers and natural fibers. Bacteria cellulose is one of the biocomposite that synthesized by micro-organism that has received the great deal of attention in recent decades (Eichhorn *et al*., 2009).The wide-ranging used of the biocomposite are include in the production of biomedical composites for gene delivery, tissue engineering and bone tissue engineering application (Svensson *et al*,.2005; Bodin *et al*., 2005)

1.1 Problem Statement

According to Czaja *et al*., (2006), unlike others product on the market, bacterial cellulose has all the ideal characteristics that need by wound dressing properties such as lower in cost , able to donate moisture, prevent infection, biocompatible, flexible, conforms to almost body surface and can reduce paint. Based on that, we want to produce new wound healing product from synthesized of bacterial cellulose and also to provide the user with the most effective healing material that can fulfill the requirement for better wound healing process.

1.2 Objective

The objectives of the experiment is to fabricate the biocomposite from bacteria cellulose, polyvinyl alcohol and fragrant oil with antibacterial properties for wound healing application

1.3 Scope of Study

The scopes of study are

i. To produce the biocomposite film from bacteria cellulose, polyvinyl alcohol and fragrant oil.

ii. To characterize the biocomposite film by using FTIR, SEM and to analyze the antimicrobial effect and also water absorption.

iii. To test the effectiveness of the biocomposite film from bacteria cellulose, polyvinyl alcohol and fragrant oil to wound healing application.

1.4 Rational and Significant of study

The rational and significant of study are

i. To produce the wound dressing material that is low cost and very effective for wound healing application.

ii. To increase the uses of bacterial cellulose in biomedical application

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Wound dressing is very important process and knowledge in daily life. It shows how to deal with wound if it happens. Antibiotic, bandage and plaster are the common wound dressing that always been used by people. But the process may take long time and high cost and less effective (Czaja *et al.*, 2005). This research will focus on the fabrication of the biocomposite film by using bacterial cellulose, polyvinyl alcohol (PVA) and also fragrant oil with antibacterial effect.

2.2 Bacterial Cellulose

Cellulose is the main component of the plant cell wall. But there are some bacteria that can also produce cellulose and the cellulose produced are called biocellulose or bacterial cellulose. Bacterial cellulose is an organic compound with the formula (C_6 H_{10} O_5). *Acetobactar xylinum* is a gram negative bacterium which can produce cellulose with using glucose as a common substrate. The bacterial cellulose extracellular excretion can form aggregated fibrils which crystallize into ribbon and assemble into a cellulose mat called as a pellicle (Suwannapinunt *et al*., 2007).

There are several bacteria that can synthesize cellulose such as genera *Aerobacter*, *Acetobacter*, *Achromobacter*, *Agrobacterium*, *Alacaligenes*, *Azotobacter*, *Pseudomonas*, *Rhizobium*, and *Sarcina* (Jonas and Farah, 1998). An overview of bacterial cellulose producers is presented in Table 2.1.

Table 2.1 Bacterial cellulose producers

Genus	Cellulose structure
Acetobactar	Extracellular pellicle, Compose of ribbon
Achromobacter	Fibrils
Aerobacter	Fibrils
Agrobacterium	Short fibrils
Alcaligenes	Fibrils
Pseudomonas	Non distinct fibrils
Rhizobium	Short fibrils
Sarcina	Amorphous cellulose
zoogloea	Not well defined

(Source: Jonas and Farah, 1998)

However, the only species that can produce enough cellulose for commercial interest is *Acetobacter* and among all of the *Acetobacter* species, *Acetobacter xylinum* is extensively used in producing the cellulose. Cellulose can be produced by using the bacterium in the fermentation process of the coconut water with an N source. It can be produced in either in static or agitated condition. If its static condition is maintained, cellulose can be harvested as a pellicle while if it in agitated it form as aggregated particles. Bacterial cellulose produced by using *Acetobacter xylinum* at the air-liquid interface of coconut water is popularly known as *nata-de-coco* (Jagannnath *et al.*, 2008). This product based on coconut water is native to the Philippines and was firstly making locally in the year of 1949. Beside of using coconut water, many others substrate can be used such as cane molasses, beet molasses, pineapple skin, cheese whey permeate , high solid permeate in order to produce *nata-de-coco* (Keshk and Sameshima, 2006). The maximum thickness of cellulose was obtained at pH 4.0 with 10% sucrose and 0.5% ammonium sulphate concentration (Jagannnath *et al.*, 2008).

Because of their ultra-fine network structure, bacteria cellulose has high biodegradability and unique mechanical strange (Brown, 1992). This showed that bacterial cellulose advantage superior from plant cellulose. The chemical and physical properties of the bacterial cellulose are such as mechanical strange, crystallinity and hydrophobicity (Muenduen *et al.*, 2008).Because of the unique properties, bacterial cellulose has found of multitude of applications such as in paper industries, textile and food industries and as a biomaterial in cosmetic and medicine (Ring *et al.*, 1986).

In medical field, bacterial cellulose approved that it is very suitable for wound dressing process especially wound that cause by burn cases (Czaja *et al.*, 2006). This is because it makes the healing process become faster and less scaring. What make bacterial cellulose's is very effective for wound healing application is its water holding ability and water vapor permeability. The high water holding ability provides a moist atmosphere at the injury site, which is suitable in healing process, while the wicking ability allows sea page from the wound to be removed from the site. According to studies by Czaja *et al.* (2004), biocellulose membrane is fully compatible and manages to prevent the burn wound from excessive fluid loss, thus accelerating the entire process of healing.

Nanocomposite material from cellulose can be fabricate statically by synthesize the bacterial cellulose gel. From the studies of Yasuda *et al.* (2005) and Nakayama *et al*. (2004) the nanocomposite for biomedical application with improved mechanical strange can be synthesis by soaking the bacterial cellulose with polyacrylamide and gelatin solution. With owning the antibacterial property and hydrophilicity, they are promising material for wound healing application (Fayazpour *et al*., 2006). Moreover, BC-PVA nanocomposite has been developed for potential application of vascular implant (Charpentier *et al*., 2006). It is also suitable for soft tissue replacement and controlled released that promoted to be a good dressing and wound healing.

2.3 Polyvinyl Alcohol (PVA)

Polyvinyl alcohol is the water-soluble synthetic polymer. It has an excellent film, emulsifying and adhesive properties. Emulsifying means that it can change the complex substance into small droplet and then dissolve it. These properties make polyvinyl alcohol suitable to emulsify the bacterial cellulose film. It is also resistance to oil, grease and solvent. Beside, polyvinyl alcohol is odorless and nontoxic. The aroma barrier properties had made polyvinyl alcohol become odorless and nontoxic means it does not irritate when having contact with human skin. So, it not harmful and can be used to human skin safely. More than that, polyvinyl alcohol is fully degradable and dissolved quickly to make it as environmental free. Polyvinyl alcohol is hydrophilic, biocompatible, and flexible synthetic polymer (Ignatova *et al*., 2006; Alipour *et al.*, 2009 and Greiner, 2007)

PVA have been ideal candidates as biomaterial. This is due to their high degree of swelling, uncomplicated chemical structure, elastic in nature, nontoxic and non-carcinogenic, and also bioadhesive. Some of their application in biomedical are tissue reconstruction and replacements, cell entrapment and drug delivery, soft contact lens material, wound covering bandage for burn victims, and etc.(Hassan *et al*., 2000).

2.4 Fragrant Oil

Fragrant oil or essential oil is the concentrated volatile aromatic compound that produced by plant. Each drop of this precious liquid is extracted from many particular plant species that can be found in certain region on this world that growth in particular environment condition. The result is a very diverse type of aromatic smell that caused by their organic compound content.

Fragrant oils are extracted from many different part of plant that have oil sacs that is from the flower, leaves, stem, root, seed, wood and also bark depend to the species of plant. Essential oils can be extracted from plant organs by crushing or by distillation in a heated aqueous or alcoholic solvent, and their active components subsequently isolated and characterized using HPLC and gas-liquid chromatography.

Most of the essential oils have naturally antibacterial properties and varying of physical and emotional effect, such as stimulation, pain relief, relaxation and also healing. The uses of the fragrant oil by people have been for so long for various applications such as perfumes, flavors, and medicine. In the new modern era, fragrant oil has been used in perfumes, cosmetics, soaps, foods, confectionary, preservatives, insect repellents, oral health care and pharmaceutical product.

For medical application, because of many of fragrant oils possess antimicrobial, antiparasitic, anticancer and other medicine properties; they have been used in many medical field. Aromatherapy is one of the uses of fragrant oil that can stimulate the sense and give the relaxation to their practitioners. Besides, they also can fight infection, contain hormone-like compound, and initiate cellular regeneration and also act as chemical defense against fungal and viral. The medicinal properties of fragrant oil have been increasing the attention over the past 20 to 30 years but still less than 10% of approximately 250000 of the world's flowering plant species have been analyzed for their pharmacological properties. Almost 25% of active, medical compounds currently prescribed in the USA and UK are isolated from higher plant (Anthony *et al.*, 2005).

Moreover, fragrant oil has a structure that similar to some compound that found in the blood and tissues. These make them compatible with human physiology. So, the uses of essential oils as the wound healing is very suitable with their properties where it can fight the infection as it is a naturally antimicrobial and also it the healing quality where it can initiate cellular regeneration. Besides that, fragrant oil also gives effect on this film because the properties of the oil itself are antimicrobial (Manuela *et al*., 2010).

2.5 Analysis Equipment

2.5.1 Fourier Transform Infra-Red (FTIR)

Fourier Transform Infra-Red (FTIR) is the analytical technique that has been used to obtain the infrared spectrum of absorption. The FTIR collect the data in the wide spectral range which measure the intensity of the narrow range of the wavelength at a time. The aim of the FTIR is to measure the effectiveness of the sample to absorb the light at each wavelength. Such element likes Si, O, H, C and N; all of them are absorb the light at the different wavelength. FTIR has been used to identify unknown materials the quality or consistency of a sample and also determine the amount of components in a mixture (ThermoNicolet, 2001).

2.5.2 Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) is a high resolution surface imaging technique and a type of electron microscope that using electron beam to scanning the sample. The electron then interacts with atom of the sample and produces the signal that will show the information about the structural topography of the sample, composition and also their properties. The types of signal that produced by SEM were secondary electrons, back-scattered electrons (BSE), transmitted electron, specimen current and X-ray. The Specimen that needs to use SEM normally required being completely dry. Many biological process and structures occur at surfaces and if antibodies are available, their components can be located within the surface structure. This is usually done in a similar way to immuno-fluorescence, using an unconjugated primary antibody followed by a tagged secondary antibody against the primary. A SEM can provide a wide range of magnification, from 30× to 100,000×, and the SEM chamber is large to work with (Li M *et al.*, 2005 and Liu *et al.*, 2009).